Effect of Ethanol and Ethanol Biodegradation Products on Prospects for Natural Anaerobic Biodegradation of Benzene at Gasoline Spill Sites

United States Environmental Protection Agency

Cherri Adair and John T. Wilson
U.S. EPA / ORD / NRMRL / GWERD

Hal White
U.S. EPA / OSWER / OUST / CRD

Robert L. Howard
Virginia Department of Environmental Quality

David Forrer
Virginia Department of Environmental Quality

Cherri Adair I adair.cherri@epa.gov I 580-436-8969

Background

There has been an increasing use of biofuels (ethanol in particular) in the fuel supply nationwide, and an increase in the number of stations that sell gasoline that contains more than 10% ethanol. The U.S. EPA needs to understand the fate of these materials if they are released from underground storage tank systems.

A release of biofuels to ground water can have undesirable consequences. Recent field demonstrations have shown that ethanol can inhibit the natural anaerobic biodegradation of BTEX compounds, causing the plume of BTEX compounds to be larger than they otherwise would be. The ethanol is biologically degraded to acetate and molecular hydrogen. These substances can accumulate to concentrations that make the anaerobic biodegradation of BTEX compounds impossible.

U.S. EPA/ORD has conducted a laboratory microcosm study and two field studies that compare the anaerobic biodegradation of ethanol in aquifer sediment from a fuel spill site to the production of molecular hydrogen and acetate.

Methods

Laboratory Studies

Laboratory microcosm experiments were conducted using sediment from an old spill of JP-4 jet fuel at the U.S. Coast Guard Support Center, in Elizabeth City, North Carolina. This sediment was well acclimated for anaerobic biodegradation of fuel components.

Figure 1. Microcosms used to study the anaerobic biodegradation of ethanol in sediment from a fuel spill site.

Microcosms were constructed in July 2009. The microcosms were constructed in 160 mL serum bottles. They contained 40 mL of wet sediment (approximately 96 grams) plus 5 mL of water, with 110 mL of gas headspace (left side microcosm in Figure 1). The microcosms that were amended with ethanol were respiked at a higher concentration of ethanol in January 2010.

All microcosms were constructed and sampled in a low oxygen glove bag. The bag was purged with Nitrogen and contained no molecular Hydrogen (Figure 2). Microcosms were incubated in the dark at room temperature on a roller.

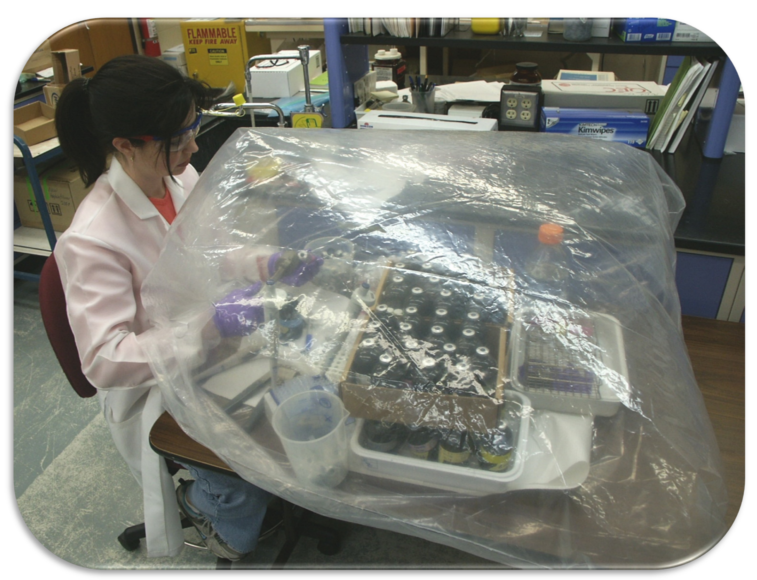

Figure 2. Construction and sampling of microcosms. Work was done in an oxygen free environment. Microcosms were not exposed to molecular hydrogen during construction or sampling.

The microcosms were sampled periodically. The concentration of molecular hydrogen and methane was determined in the gas headspace of the microcosms. The concentrations of ethanol, acetate and butyrate and the pH were determined in the pore water of the microcosms.

Field Studies

Ground water samples were collected from conventional monitoring wells. The concentration of molecular hydrogen, methane, ethanol, acetate and butyrate and the pH were determined in the ground water samples.

Pathways of Anaerobic Biodegradation

Ethanol is fermented by acetic acid bacteria to acetic acid and molecular hydrogen as described in the reaction below.

$$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2\ H_2$$

Under anaerobic conditions, acetate is further fermented to carbon dioxide and methane.

$$CH_3COO^- + H^+ \rightarrow CO_2 + CH_4$$

Hydrogen and bicarbonate can be fermented to acetate and water.

$$2\ HCO_3^- + 1\ H^+ + 4\ H_2 \rightarrow CH_3COO^- + 4\ H_2O$$

Acetate and hydrogen can be fermented to butyrate and water.

$$2\ CH_3COO^- + H^+ + 2\ H_2 \rightarrow CH_3CH_2CH_2COO^- + H_2O$$

These last two reactions are reversible depending on concentrations of reactants and products.

Effect of Degradation of Ethanol on the Energy Available from Anaerobic Biodegradation of Benzene

The energy available from a chemical reaction can be calculated by comparing the energy required to make the reactants and the energy required to make the products. The change in energy between the reactants and products is the energy released (or consumed) in the reaction. The Gibbs Free Energy (ΔG') is a quantitative estimate of the energy that is available to microorganisms that carry out a particular reaction.

The values of ΔG' depend on the energy content of reactants and products, and the concentrations of reactants and products. If the concentrations of reactants are low, and the concentrations of products is high, the value of ΔG' is high.

If ΔG' is positive, the reaction requires energy. Water does not run up hill, and bacteria cannot gain energy to grow with a positive ΔG'. If ΔG' is more negative than -20 kJ/mole, generally bacteria can grow. If ΔG' is between 0 and -20 kJ/mole, bacteria may or may not be able to grow, depending on the strain of bacteria.

Both ethanol and benzene are fermented to acetic acid and molecular hydrogen. If ethanol is available in the water, the fermentation of ethanol can produce so much hydrogen and acetate that the fermentation of benzene is not possible.

$$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2\ H_2$$

$$C_6H_6 + 6\ H_2O \rightarrow 3\ CH_3COO^- + 3\ H^+ + 3\ H_2$$

Results

Laboratory Studies

Figure 3A presents the time course of biodegradation of ethanol in one of the microcosms. The rate of degradation of ethanol was linear (zero order) with time. As ethanol was consumed, acetate and butyrate were produced and accumulated in the water. When the ethanol was entirely consumed, then butyrate and acetate were depleted. The butyrate was converted back to acetate, and the acetate was converted to methane. After 280 days, all the carbon from the ethanol was converted to methane and carbon dioxide.

Figure 3B compares the time course of production and consumption of molecular hydrogen in the microcosms. As long as ethanol was available in the water, the degradation of the ethanol sustained high concentrations of molecular hydrogen.

Figures 4A and 4B compare the concentration of ethanol in the microcosms to the value of ΔG' for anaerobic biodegradation of benzene. In the red zone in the figures, the value of ΔG' is positive and benzene will not be degraded. In the yellow zone the value of ΔG' is between 0 and -20 kJ/mole, and benzene probably will not be degraded. In the green zone the value of ΔG' is more negative than -20 kJ/mole, and benzene can be degraded.

As a general rule, when the concentration of ethanol was above 4 mg/L, the concentrations of molecular hydrogen, acetate and H^+ were too high to allow anaerobic degradation of benzene.

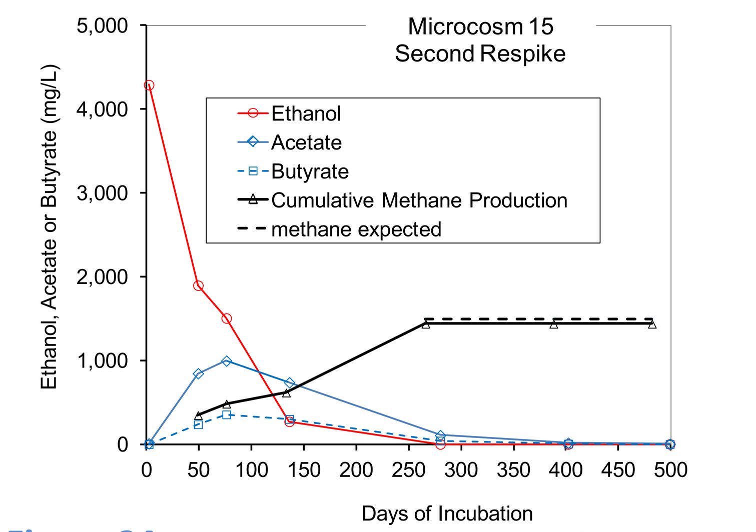

Figure 3A. Consumption of ethanol and production of acetate, butyrate, and methane in a microcosm.

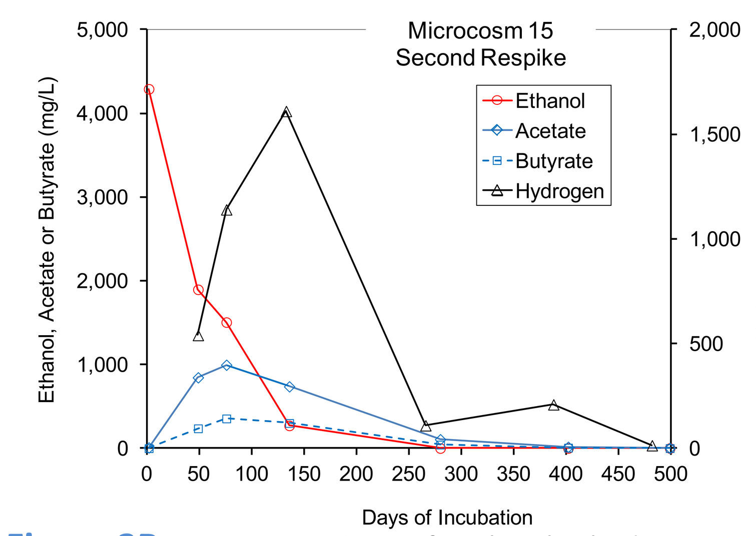

Figure 3B. Concentrations of molecular hydrogen are high as long as ethanol is available to be degraded to produce molecular hydrogen.

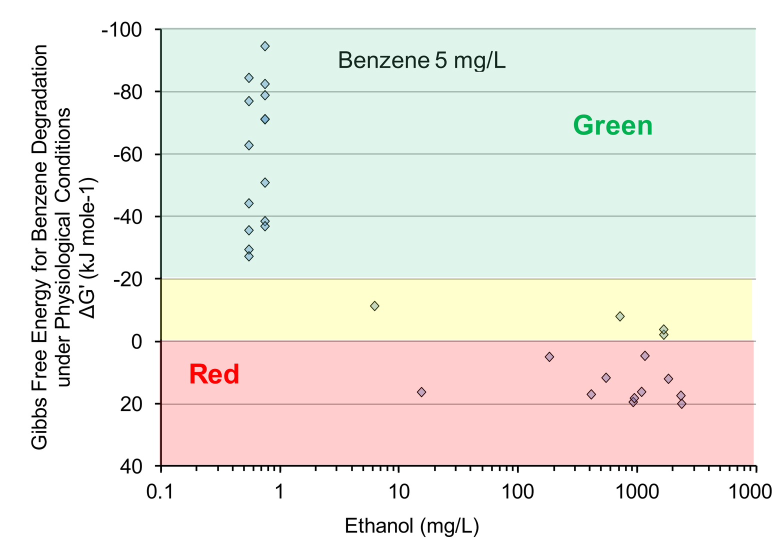

Figure 4A. Relationship between the concentration of ethanol in the microcosms, and the thermodynamic feasibility of anaerobic biodegradation of benzene at a concentration of benzene in water that is typical of an un-weathered spill of gasoline.

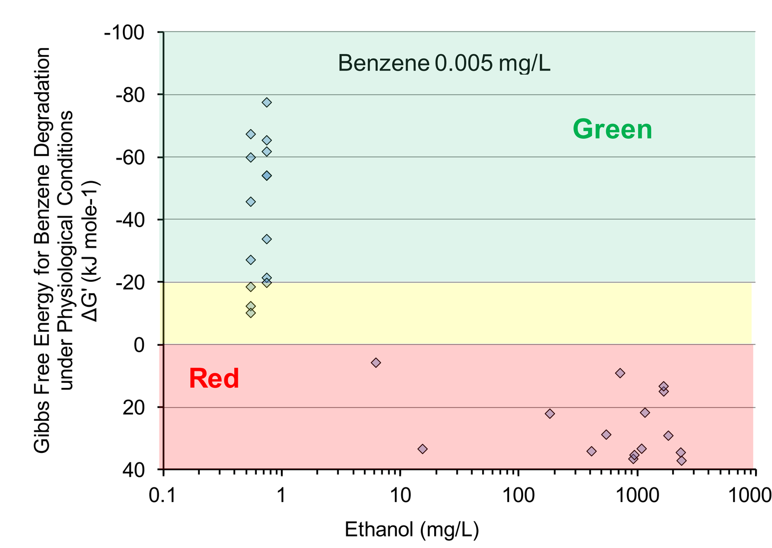

Figure 4B. Relationship between the concentration of ethanol in the microcosms, and the thermodynamic feasibility of anaerobic biodegradation of benzene, when benzene is present at the MCl.

Field Studies

Figure 5 presents the location of monitoring wells at a spill of gasoline containing 10% ethanol (E10). The ground water was in fractured limestone. Table 1 presents data on the concentrations of ethanol in monitoring wells that ringed the pit containing the failed UST. The concentrations of ethanol were much lower one year after the spill, in part due to biodegradation of the ethanol, in part due to a pump and treat system installed at the site.

Table 2 compares the concentrations of ethanol and its degradation products. In wells with measurable concentrations of ethanol, the concentrations of ethanol degradation products were high, the values of ΔG' were positive or just barely negative, and bacteria could not use benzene to grow. As would be expected, the concentration of benzene in these wells was high.

Figure 6 presents the location of monitoring wells at a spill of E95. Table 3 compares concentrations of ethanol and ethanol degradation products in ground water from the wells. As was the case for the E10 spill site, when the concentrations of ethanol were high, the values of ΔG' were positive or just barely negative, and bacteria could not use benzene to grow. Again, the concentrations of benzene in the presence of ethanol were high.

Table 1. Concentrations of ethanol in monitoring wells at the spill site in Figure 5.

	12/10/2010	4/25/2011	9/13/2011
	Day sincenew release reported		
	59	195	336
	Ethanol (mg/L)		
W-8	205,000	596	393
W-6	24,800	1,112	79
W-1	9,270	426	2,930
W-7	7,740	0.563	<0.1
W-9	3.73	<0.1	<0.1
W-10	1.67	<0.1	<0.1
W-5	<0.1	<0.1	<0.1
W-2	<0.1	0.428	36

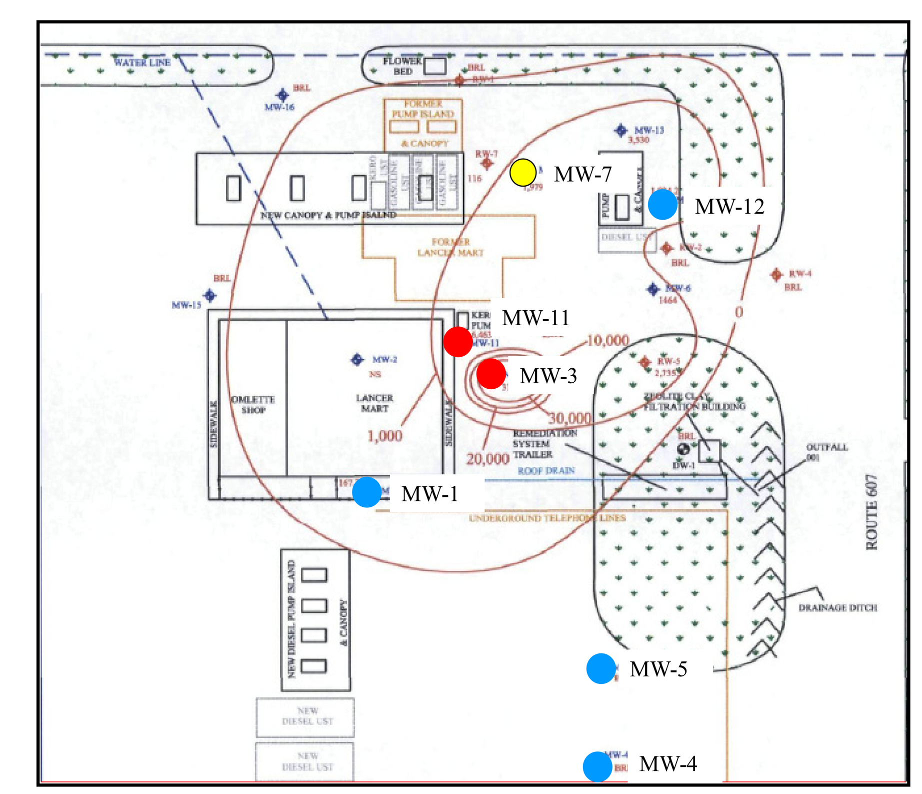

Figure 6. Locations of monitoring wells at a spill of E95.

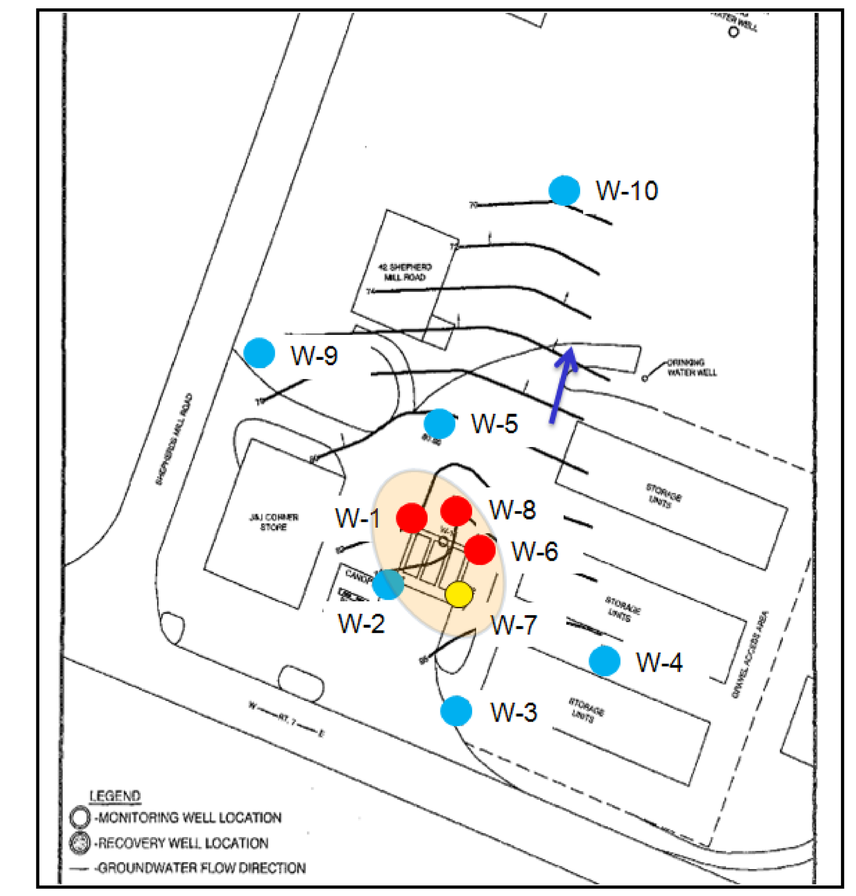

Figure 5. Locations of monitoring wells at a spill of E10.

Table 2. Comparison of the concentrations of ethanol in ground water at the E10 spill site in Figure 5, to concentrations of benzene, molecular hydrogen and acetate.

Well	Date	Ethanol	Benzene	Hydrogen	Acetate	ΔG' Benzene
		mg/L	mg/L	ppm	mg/L	kJ/ mole
W-8	4/2011	569	1.4	85,000	189	14
W-6	4/2011	1112	7.8	171,000	194	11
W-1	4/2011	462	1.1	141,000	441	17
W-7	4/2011	0.56	2.3	157,000	19.5	-4.1

Table 3. Comparison of the concentrations of ethanol in ground water at the E95 spill site in Figure 6, to concentrations of benzene, molecular hydrogen and acetate.

	Ethanol	Benzene	Acetate	pH	Hydrogen	ΔG' Benzene
	mg/L	mg/L	mg/L		ppm	kJ/mole
MW-3	6130	8.50	269.0	4.9	64634	59
MW-11	930	2.00	310.0	5.2	4947	40
MW-7	19.2	0.33	28.4	6.1	476	-6
MW-4	0.8	0.0011	<0.1	6.6	342	-45
MW-5	0.2	0.00069	<0.1	5.1	<32	-42
MW-12	<0.1	0.34	<0.1	6.3	182	-58
MW-1	<0.1	0.09	<0.1	6.5	527	-52

Summary and Conclusion

The data from the field sites was consistent with the laboratory data. When concentrations of ethanol were above approximately 5 to 20 mg/L, the concentrations of ethanol biodegradation products would not permit the anaerobic degradation of benzene.

Disclaimer